

MICROBIOLOGICAL STUDY OF IMITATION AND  
FILLED DAIRY PRODUCTS AND THEIR COMPONENTS

by

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## INTRODUCTION

Milk has always been one of man's most perfect foods since it is an excellent source of proteins, fat, calcium, phosphorus, and Vitamin A, and until recent years was in no danger of being replaced in the human diet. A number of years ago filled and imitation milks began to appear in markets.- the first states to allow the sale of such products being Arizona and California (Tuckey, 1968).

When it appeared that these filled and imitation products would gain great acceptance, nutritionists began to examine these products to see how they compare to milk and milk products. A great deal has been learned so far concerning the nutritional status of filled and imitation milk, but very little work has been done to check the microbiological quality of these products.

The purpose of this study was to examine as many of these imitation and filled products as possible to determine their microbiological quality. Since components of these products could be a source of contamination, it was decided that the components used to fabricate these products should be examined also.

## REVIEW OF LITERATURE

### Imitation Products

In the past three or four years imitation milk and other imitation dairy products began to make inroads on the dairy market (Saal, 1967). In 1966, (Miller, 1968), the total market for vegetable-fat dairy products was in excess of \$240 million at retail prices and a 1967 growth rate of 36% was anticipated. Early in 1968 (A. Dairy Rev., 1968), dairy leaders saw the imitations as the biggest problem the dairy industry would have to face that year.

There are many reasons for the growing acceptance of these imitation products (Miller, 1968). One is the long shelf life of the finished products. Some of these products are purchased frozen and keep for several weeks under refrigeration. Coffee whiteners in a powder form can be kept indefinitely without refrigeration. The low cost of basic ingredients of imitation products and consequently their lower market price also aids a great deal in their acceptance. Improved technology in the development of new lipid systems enables the processors of vegetable fats to produce fats that meet almost any desired specifications. Since the dairy industry by law cannot mix vegetable fat and milk fat, it is limited in the development of new lipid systems.

### Definitions

Imitation products now on the market fall into two categories - filled milk products and imitation milk products (Dairy Council Digest, 1968) (Kosikowski, 1968). Filled milk is defined as a product made

by combining fats or oils other than milk fat with milk solids so that the resulting product is an imitation or semblance of milk, cream, or low fat milk, whether or not condensed, evaporated, concentrated, dried, or desiccated (Holland, 1968). The types of milk solids specified in the Federal Filled Milk Act are "any milk, cream, or skimmed milk whether or not condensed, evaporated, concentrated, powdered, dried, or desiccated" (Adm. Reports, FDA).

At present there are two types of filled milk being sold (Dairy Council Digest, 1968). One is a combination of fluid skim milk with or without skim milk solids and a vegetable fat made in semblance of milk. The second type basically contains water, non-fat dry milk, vegetable fat, and an additional source of protein such as soy protein or sodium caseinate.

In contrast to filled products which contain non-fat milk derivatives, imitation products do not contain any ingredients which are considered dairy products. Products classified as imitation milks contain such ingredients as water, corn syrup solids, sugar, vegetable fat, a source of protein such as sodium caseinate or soy protein, made in semblance of milk.

#### Legal Status of Imitation and Filled Products

The production of filled and imitation milks is governed by state and federal regulations. However, as a general rule, they are not subjected to sanitation and composition regulations as rigid as those imposed in the production and processing of Grade A dairy products. The Federal Filled Milk Bill (Adm. Reports), which was passed in March,

1923, prohibits interstate traffic in milk or cream containing any fat other than milk fat. In contrast to the filled milks, imitation milks do not fall under the provisions of the Federal Filled Milk Act and can be shipped in interstate commerce. As late as September 1, 1967, there were no provisions in any Federal Milk Marketing Orders dealing specifically with imitation fluid products (Saal, 1967).

In a survey of states only ten states (of 42 responding) permit the processing and selling of filled milk products while the processing and selling of all types of imitation products is permitted in 32 states of 40 responding (Amer. Dairy Rev., 1968). In Kansas, filled products cannot be sold or processed in milk plants while imitation products can be processed and sold.

#### Nutritional Value of Imitation and Filled Products

Since these less expensive imitation and filled products might be considered replacements for milk in the diet, especially for infants and children, a great deal of work has been done on the nutritive value of these products.

The vegetable fat most commonly used in imitation and filled products is hydrogenated coconut oil, (Dairy Council Digest, 1968), an oil high in lauric acid. Several investigators, (Nielsen, 1968) Brink, 1968), have found lauric acid to have a powerful effect in raising the serum cholestreol level, but is not yet been found conclusive. Also it has been found that coconut oil in the diet is poorly tolerated by children (Brink, 1968)(Rice, 1960)(Smith, et. al., 1942).

Sodium caseinate and soybean protein are protein sources most



often used, (American Dairy Review, 1968)(Kosikowski, 1968)(Nielsen, 1968), and while they are good protein sources they are not nutritionally equivalent to native milk proteins. Although sodium caseinate is made from milk casein it is not regarded by regulatory agencies as a dairy product or milk ingredient but as a chemical or food additive generally regarded as safe (GRAS) for human consumption, (Nielsen, 1968). Non-fat dry milk solids used in filled products are inexpensive, excellent protein and essential amino acid sources, contain water-soluble vitamins, and the minerals calcium and phosphorus (Krauss, 1947)(Dairy Council Digest, 1968).

#### Bacteriological Quality

So far very little work has been done concerning the bacteriological quality of imitation and filled products. An imitation milk was examined for total plate count and coliform (Kosikowski, 1968). The standard plate count was 155,000/ml and the coliform count was 120,000/ml. These counts are considered excessive by present day public health standards and regulations applied to pasteurized cows milk. The standard proposed for pasteurized certified milk by the American Association of Medical Milk Commissions, Inc. prescribes that coliform counts shall not exceed 1/ml, and the standard plate count shall not exceed 500/ml.

#### How to Make Imitation Milk

The ingredients in imitation milks, coffee whiteners, and whipped toppings are all similar with appropriate adjustments in amounts.

The typical ingredients in a liquid coffee whitener would be as follows:

<u>Ingredients</u>	<u>%</u>
Vegetable fat	8-12
Protein material	1-2
Emulsifiers	0.2-1.0
Buffers	0.1-0.25
Stabilizers	0.02-0.15
Flavors & color	
Sweetening agents	5-10
Water	Remainder

The steps in the manufacture of most dairy substitutes are quite similar (Holland, July, 1968)(Miller, 1968). The protein material, buffers, stabilizers and sweetening agents are dry mixed and then added to the water. After mixing well to disperse the solids the mixture is heated to 43.3 C and the fat, emulsifiers, and color are added. It is then heated to 71.7 C for 30 minutes. Flavor is added and the mixture is homogenized through a two-stage homogenizer at 176 and 35 kg/cm<sup>2</sup> for the first and second stage respectively. The mix is cooled as rapidly as possible to 4.4 C, and if desired frozen as rapidly as possible to -18 C. Mix is defrosted at 4.4 C before use. For powdered products, such as coffee whiteners, the water is removed by spray drying.

#### NON-FAT DRY MILK

Since non-fat dry milk is an important ingredient in filled milk products, mention should be made of its uses and standards. Non-fat dry milk is of such quality that it can be used for human consumption whereas dry skim milk is used for animal feed.

Drying is one of the oldest methods of preserving food known, and dry milk, especially non-fat dry milk, makes it possible to supply



the nutrients in milk to areas where there is no dairy industry or where economic conditions place fresh milk out of reach of many. Dried milk products also are widely used because of the ease with which they are preserved, and the conservation of transportation, facilities and storage space due to reduction in bulk. The keeping quality of dried milks depends primarily on their low moisture content, for without adequate water there can be no microbial metabolism and therefore no microbial spoilage. Chemical spoilage can occur, however, in the form of non-enzymatic browning or chemical changes brought on by high temperatures during storage or during storage for a long period. Dry whole milk has limited keeping qualities because of the high fat content and thus far no effective antioxidants have been found.

In the period from 1947-1961, the use of non-fat dry milk solids increased nearly 90% - more than the use of any other dairy products. Non-fat dry milk is used by the baking industry in bread, in prepared pancake, waffle and biscuit mix, in ice cream, infant foods, cocoa and chocolate drinks, malted milk, meat products, and many cat and dog foods. Nearly all dry whole milk and about 80% of non-fat dry milk is made by a spray-dry process (Lampert, 1965). In 1963, 2.1 billion pounds of non-fat dry milk were produced in the United States - 2 billion of which was spray-dried.

#### Standards

Non-fat dry milk contains not more than 5% moisture or 1.5% fat (Foster, et. al., 1957). USDA standards vary from 1.25 to 1.5% fat and 4-5% moisture depending on the grade.

The American Dry Milk Institute and USDA have recommended two

grades of non-fat dry milk, "Extra" and "Standard", (Foster, et. al., 1957). To receive a grade of "Extra" the total bacterial count must not exceed 50,000/gram regardless of whether drying is done by spraying, vacuum or roller methods. For "Standard" grade the total bacterial count must not exceed 100,000/gram.

Neither the USDA nor the American Dry Milk Institute has proposed coliform standards for non-fat dry milk.

### SALMONELLAE IN FOODS

#### In Dry Milk and Other Foods

Active surveillance of milk drying plants for the presence of salmonellae in the plants and products was initiated in 1966 (Sal. Surv., 1967). In 1967, dried milk accounted for 575 salmonellae isolations from non-human sources (6.5%) compared with 271 isolations (3.5%) in 1966. No isolations were reported from this source prior to 1966.

A report of the Dairy Division, Consumer and Marketing Service, USDA, (Nielsen, 1967), released early in 1967, revealed that, based on a survey involving 5884 samples from 214 plants, 13% of the plants had Salmonella-positive samples and 19% of the plants had Salmonella-positive environmental samples. In 1968, the USDA tested product and environmental samples from 210 milk drying plants in 29 states. In 17,496 product samples 39 (0.22%) were positive and of 3,142 environment samples 152 (4.8%) were salmonellae positive (Grabe, 1968).

During 1966, several non-fat dry milk products were withdrawn from markets due to salmonellae contamination (Sal. Surv., 1966).

In 1968, an outbreak of salmonellosis was reported in families that had purchased and consumed a particular brand of powdered skim milk (Severs, et. al., 1968). A check of open boxes in the homes and unopened boxes of the same batch number purchased at markets revealed all were positive for Salmonella newport.

Non-fat dry milk may be mixed with other ingredients and sold in this way - thereby widening the possible spread of salmonellae contaminated products (Potter, 1967). Instant puddings, desert mixes, and canned frosting are some of the products containing non-fat dry milk which are frequently reconstituted and consumed without cooking or given further heat treatment. When these products are contaminated they are of particular concern.

Although plants producing dried milks have come under surveillance only in the last three years, cases of salmonellosis attributed to fluid milk, usually raw milk, have been reported for many years (Bcwner, 1965). Food poisoning attributed to salmonellae in milk and milk products does not occur often, but some of these outbreaks can be quite explosive. In England and Wales raw milk was often sold directly to the public or to a school for lunchroom use (Parry, 1962)(McCall, 1953)(Know, 1963). In one instance (McCall, 1953) raw milk supplied to schools and the public caused an explosive outbreak affecting 610 people. The etiologic agent was found to be Salmonella dublin. In this outbreak as well as in others (Schroeder & Dale, 1960) salmonellae were isolated from cows in the herd implicating them as the source of infection. Localized outbreaks have also been traced to a person who supplies raw milk to neighbors (McCroan & McKinley, 1962).

Outbreaks of salmonellosis food poisoning also have been traced

to bakery products (Harvey, 1961)(Agate, et. al., 1961)(Reardon & Fuimora, 1963), and the environment in the bakery (Harvey & Phillips, 1961). A comparison of these studies shows that contamination from workers and not from the ingredients was most often the cause of contaminated products. Certain kinds of bakery confectionary are ideal culture media for salmonellae and the potential risk of contaminated products would be great due to poor personnel hygiene on the part of workers.

#### Potential Sources of Contamination

Potential sources of contamination of milk and milk products are nearly limitless (White, 1967)(Potter, 1967)(Hedrick, 1967). The primary sources of salmonellae are man and animals, and from them organisms can spread to equipment, air, water, and packaging materials used in producing dry milk and non-fat dry milk. Flies, cockroaches, rodents and birds also may be possible sources of contamination.

The major steps in producing non-fat dry milk are (1) separating skim, (2) pasteurizing, (3) concentrating, (4) reheating, (5) spray drying, (6) instantizing, (7) cooling, and (8) packaging (Potter, 1967). If milk entering the plant is contaminated, separation of the cream will remove some of the organisms while pasteurization should completely inactivate all common milk-borne pathogens including salmonellae. A check should always be maintained to see that pasteurization controls insure "positive pasteurization".

If, however, after pasteurization, there is opportunity for salmonellae contamination, as there frequently is, then none of the

subsequent steps as currently practiced can be counted on for total destruction of this organism. In spray drying, a fine spray of milk is forced rapidly through a stream of air heated to 65.5 C - 193 C. Nearly all non-fat dry milk is then instantized to make it dissolve more rapidly.

#### Heat Resistance

Salmonella are not particularly heat resistant and proper pasteurization should kill all salmonellae in milk. A heat resistant strain of Salmonella senftenberg 775W has been isolated from dried egg (NG, et. al., 1969)(Thomas, 1966), and a great deal of work has been done to see if this organism would survive pasteurization as recommended by 1965 Pasteurized Milk Ordinance of the U.S.P.H.S. (Read, et. al., 1968). Providing the Salmonella concentration of milk does not exceed a calculated  $3 \times 10^{12}$ /ml, the present milk pasteurization process as recommended by the Public Health Service will inactivate S. senftenberg 775W (Read, et. al., 1968).

The heat resistance of salmonellae in dried milk has been studied also (McDonough and Hargorve, 1968). Artificially contaminated powders of various moisture levels were subjected to tests which included storage at temperatures of 4.4 - 50 C, storage with chemical additives, exposure of thin layers to oven heat ranging from 60 - 115 C, boiling of fixed beds of powder with hot air streams at 87.7 and 148.8 C, and determination of time-temperature necessary for destruction of salmonellae in fluid milk and concentrated milks. Moisture levels and storage temperatures influenced growth and survival, and Salmonella added to non-fat dry



milk were quite resistant to dry heat.

In the Food Service Sanitation Manual of the U.S. Department of Health, Education and Welfare, it is stated that foods shall be maintained at temperatures above 60 C or below 7.2 C at all times except during processing since these temperatures will presumably preclude the growth of salmonellae and other food poisoning organisms (White, 1967). However, Angelotti, (Angelotti, et. al., 1961) found that temperatures of 5.56 C or below were necessary to prevent growth of salmonellae and staphylococci in perishable food.

Food poisoning outbreaks often are attributed to foods which are prepared and held before serving or are placed on a steam table while being served. If holding temperatures are not high enough, organisms present will be able to grow.

Mixed cultures of salmonellae and staphylococci were cultured in custard, chicken a la king, and ham salad for 24 hours at temperatures from 44.4 C to 49 C and at 35 C (Angelotti, et. al., 1961). Good growth of test organisms was observed in all three foods at 35 C. The results indicate that the temperature growth range for salmonellae and staphylococci in foods of the type studied is 6.67 C to 45.5 C and holding perishable foods is to be avoided in the midpoint of this range for periods that would permit the growth of salmonellae and staphylococci.

#### STAPHYLOCOCCI IN FOODS

Staphylococci also are often incriminated in outbreaks of food poisoning, especially in foods such as eclairs, pastry filling, cream



pies and salad dressing. Most foods responsible for staphylococci food poisoning outbreaks often are not cooked after preparation, and if allowed to stand in a warm place for 6-8 hours contaminating organisms will grow and enterotoxin will be produced.

A great many surveys of possible staphylococci contamination of products purchased directly from markets have been done also. One hundred vanilla malted milk shakes were analyzed for incidence of staphylococci (Foltz & Mickelsen, 1964). Ten were found to contain coagulase-positive, phage-typable strains of staphylococci. In precooked, frozen, desert-type foods (Verma, et. al., 1964), 28 of 102 showed staphylococci contamination although none were coagulase positive. (Foltz, et. al., 1960) examined 207 samples of pasteurized dairy products obtained from consumer marketing channels for the presence of staphylococci. Cheese also has been found to contain staphylococci (Mickelsen, et. al., 1961); in 120 samples 7.2% were possible sources of coagulase positive staphylococci.

#### In Spray Dried Milk

Staphylococci food poisoning also has been associated with spray-dried milk (Ander & Stone, 1955). The incriminated food in five separate outbreaks was an artificial cream in which dried milk was an ingredient. S. aureus was isolated from non-fat dry milk and from the prepared cream. Most products had not had long enough incubation to allow good growth of staphylococci, and the accompanying production of toxin indicated the milk must have been contaminated before being sealed in tins.

Formation of enterotoxin during the production of spray-dried

milk is likely to occur at two stages:

1. Before the milk has received heat treatment. Staphylococci enterotoxin is thermostable requiring a temperature of 100 C over a considerable period for complete destruction. Thus, toxin formed in raw milk would not necessarily be destroyed by the temperatures reached in processing and might well be present in the final product.
2. After preheating and evaporation of milk and before spray drying re-infection may occur. Any delay in processing will permit multiplication of organisms and production of toxin.

#### In Imitation Cream Fillings

Many manufacturers of imitation cream fillings give the impression these products are safe for use without refrigeration regardless of temperature, and consequently vendors, bakers, and food service operators in general are becoming extremely careless with these products. Contrary to manufacturers belief, food poisoning staphylococci grow very well in these imitation cream products (McKinley & Clarke, 1964)(Post, et. al., 1961).

Imitation cream-filled doughnuts obtained from a vending machine caused several cases of food poisoning (McKinley & Clarke, 1964). It was found that the temperature in the vending machine had been exceptionally high, but the manufacturers said since the cream was an imitation refrigeration was not necessary.

A synthetic cream filling consists basically of starch, sugars

(sucrose and dextrose), sodium chloride, natural and artificial flavorings, emulsifiers, stabilizers, preservatives and other chemical additives. These are made available to bakeries under the claim that they are not capable of supporting bacterial growth and pies made with such filling can be marketed without refrigeration.

Seven synthetic cream fillings were examined for their ability to support multiplication of added Staphylococcus aureus and their normal flora at room temperature (Crisley, et. al., 1964).

When prepared with water, all seven supported multiplication of bacteria present, largely bacilli, while five showed a decrease in staphylococci count. Substitution of milk for water in preparing the fillings plus addition of minute amounts of whole egg and combination with pie crusts increased the ability of the fillings to support staphylococci multiplication.

Pies made with synthetic fillings rehydrated with water supported profuse staphylococci growth to the extent that they might be hazardous when held at room temperature before being sold.

#### MATERIALS AND METHODS

Samples tested in the survey were obtained from two main sources. Finished products were purchased from various grocery stores around Manhattan, and in the case of frozen and liquid products were examined within 24 hours. Samples purchased commercially consisted of frozen and powdered whipped topping, liquid and powdered coffee whiteners, cereal topping, imitation whipping cream, aerosol whipped topping, puddings, and frosting. Several pasteries with imitation cream fillings

and individual tetrapack coffee whiteners purchased from local restaurants also were examined.

Since it is quite possible for bacterial contamination to enter a finished product through one of the components it was decided as many of these ingredients should be checked as possible. Mr. Mickelsen wrote to companies manufacturing these ingredients requesting samples for use by the Dairy Science Department and nine companies responded with a total of thirty-one samples.

#### Sampling

Samples were received from manufacturers packaged in sealed cans or plastic bags. Using sterile tongue depressors or tablespoons, samples were removed aseptically and placed in sterile whirl-pack bags. Samples were stored in the refrigerator until bacteriological examinations were carried out.

#### Isolation Methods for Salmonella

Historically, the first procedures for isolation of salmonellae were for clinical use. Often in clinical specimens isolation can be made from direct plating on solid media, but liquid enrichments also are used to recover salmonellae present in small numbers. A good enrichment must encourage the growth of salmonellae and at the same time suppress or inhibit the growth of other gram-negative organisms such as coliforms.

Tetrathionate +  $I_2$ , tetrathionate + BG +  $I_2$  (Read & Reys, 1967), and selenite broth are effective enrichment media, but with food products in which the number of salmonellae may be small pre-enrichment aids

detection, (Taylor, 1961). Pre-enrichment stimulates organisms to grow from a state of reduced viability and physiological inactivity suffered during processing and/or storage (Adinarayanan, et. al., 1965) (North, 1961). After pre-enrichment a larger number of organisms, in a state of active growth will be transferred to the selective medium with a correspondingly better chance for multiplication. Lactose has great value as a pre-enrichment (North, 1961) (Taylor & Sillicker, 1961) in that it restores salmonellae to a state of active growth. In the presence of a mixed flora, fermentation of the lactose results in a lower pH which serves to hold in check other types of micro-organisms. This pH change does not appear to be sufficient to affect the growth of or be lethal for salmonellae. Pre-enrichment is further enhanced by uniform dispersal of material in the media. Fatty foods do not disperse easily in pre-enrichment media, but the addition of a wetting agent (Tergitol # 7) helps disperse fatty food and increases the efficiency of detection of Salmonella (Galton, et. al., 1954) (Hall, et. al., 1964).

The use of 0.002% brilliant green or 0.004% crystal violet in reconstituted non-fat dry milk or fluid milk permits the unrestricted growth of salmonellae and coliform bacteria when small numbers of these are present (North, 1960).

Many different plating media have been used for isolation of salmonellae. Counts are consistently higher on brilliant green, bismuth sulfit, eosin-methylene blue, desoxycholate agar or cystine selenite (Taylor, 1958). Brilliant green is more inhibitive for enteric organisms other than salmonellae and by addition of sodium sulfadiazine to brilliant green pseudomonads are effectively inhibited (Galton, et. al., 1968).



## Isolation of Staphylococci

Staphylococci associated with food poisoning are characterized by coagulase production, mannitol fermentation and pigmentation, and the latter two characteristics are the basis for the isolation of staphylococci. Media now most frequently used are Mannitol Salt Agar, and Staph-110 media in which the selective action is due to the presence of 7.5% sodium chloride.

## Dilutions

Contents of each liquid sample were mixed well before transferring 11 grams (or 11 ml) of sample to a 99 ml sterile water blank, and dilution blanks were shaken well to disperse sample. In fatty samples 2 ml of 6% tergitol were added to aid in dispersing sample. This 1:10 dilution was then used to prepare a 1:1000 dilution.

## Plate Count

Using sterile 1 ml pipettes, 1 ml and 0.1 ml of the 1:10 dilution and 1 ml of 1:1000 dilution were placed in sterile petri dishes. Plate Count Agar (Difco), melted and tempered to 45 C, was poured into plates and medium and inoculum were thoroughly mixed. Plates were incubated at 37 C for 48 hours, and plates with 30-300 colonies were counted using a Quebec colony counter. In some cases counts were so low that plates with fewer than 30 colonies had to be counted.



### Coliform Count

One ml and 0.1 ml of the 1:10 dilution were transferred to sterile petri dishes and plates were poured with Violet Red Bile Agar (Difco). Inoculum and agar were thoroughly mixed, and after the plates hardened an overlay of 3-4 ml of Violet Red Bile was poured. The plates were incubated at 37 C for 24 hours, after which dark red colonies were counted. Isolates were streaked on Eosin Methylene Blue Agar and confirmed on basis of lactose fermentation and IMVIC reactions.

### Staphylococci Count

Plates of Mannitol Salt Agar (Difco) were poured and allowed to dry. One ml and 0.1 ml of the 1:10 dilution were transferred to the surface of dry plates and spread according to the surface plating technique of Snyder (Snyder, 1947). Plates were incubated at 37 C for 24 hours, after which yellow colonies surrounded by a yellow zone were examined by gram stain.

### Salmonella

Samples of ingredients received from manufacturers and powdered and whipped samples were examined by the following procedure. Thirty grams of food were weighed aseptically into sterile 16 ounce screw capped jars. One-hundred ml of lactose broth (200 ml if necessary) were added to obtain a good suspension. If the sample had a high fat content 6 ml of 10% solution of Tergitol # 7 were added. Samples were shaken vigorously and then incubated, with loose lids, at 35-37 C for 48 hours.

After 24 and 48 hours, lactose broth was streaked to brilliant green (BG) agar plates, and 1 ml was subcultured to 10 ml of tetrathionate brilliant green broth (TET). TET broth subculture was incubated at 35-37 C for 24 hours, and then a loopful was streaked to BG.

Liquid milk products were examined by a slightly different method. Samples were usually in pint (550 ml) or  $\frac{1}{2}$  pint (225 ml) size, and the entire sample was tested. Eleven ml of 0.1% aqueous brilliant green dye were added to 550 ml samples (5.5 ml to 225 ml) and flasks were incubated at 35-37 C for 24 hours. One loopful from each flask was streaked to BG and 10 ml was transferred into 100 ml TET broth. Plates and TET broth were incubated at 35-37 C for 24 hours. One loopful of TET broth was streaked to BG and incubated at 35-37 C for 24 hours.

#### RESULTS AND DISCUSSION

It was anticipated that these products would be low in total bacterial count, and from an esthetic and sanitary viewpoint it was hoped that coliforms, salmonellae and staphylococci would not be found.

Of 84 commercially purchased samples examined, none were found to contain salmonellae or staphylococci, and only 4 (4.7%) of these samples were found to contain coliforms. There was not a great deal of variation in plate counts although certain correlations can be made with types of products and high plate counts.

Table 1. Bacterial Count of Coffee Whiteners, Imitation Half and Half, and Cereal Toppings.

Product	No. Samples	S.P.C./gram		Coliforms/gram(ml)
		Range	Average	
Liquid Coffee Whiteners	8	0-260	45	1/ml*
Powdered Coffee Whiteners	13	0-200	50	10/ml*
Cereal Topping	3	0-1500	500	0
Imitation Half & Half	1	0	0	0
Tetrapacks	12	20-160	66	2/ml*

\* In one sample only

The coliform from the one liquid coffee whitener was E. coli, while the coliforms from the powdered coffee whitener and the imitation half and half were Aerobacter aerogenes.

Table 2. Bacteriological Content of Non-Dairy Toppings.

Product	No. Samples	S.P.C./gram		Coliform/gram
		Range	Average	
Pre-whipped	6	0-70	17	0
Aerosol	8	0-150	19	0
Powders	6	20-700	220	0
Liquid to be Whipped	12	0-800	66	0

Table 3. Bacteriological Content of Miscellaneous Foods.

Product	No. Samples	S.P.C./gram		Coliform/gram
		Range	Average	
Deserts	3	0-380	180	0
Pudding	4	0-190	78	0
Frosting	2	20-170	95	0
Pie Filling	1	0	0	0
Pastry Filling	5	0-1800	550	130*

\* In one sample only

The coliform from the pastry filling was found to be Aerobacter aerogenes.

The samples received from the manufacturers were of two types - those which were oils or fats and those which were non-fat. Fats do not encourage the growth of pathogenic bacteria (Hobbs, 1953) and the oil or fat samples were not found to have any bacteria at all, whereas the powdered samples had plate counts which ran from quite low to some that were rather high.

Table 4 shows the plate counts of the non-fat components; coliform, staphylococci and Salmonella were not found in these samples.

Table 4. Plate Count for Non-Fat Components.

Sample	S.P.C./gram
Imitation Half & Half	0
Imitation Milk	0
Sta-Rite Sour Cream Products	150*
C.G. Citadel for Filled Milk	0
Koffee White	40
Vegetable WC	0
Vegetable Sour Dip Base	40
Vegatable CM	4700
Vegatable MM	0
Domestic Sodium Caseinate	0
Beverage Base - Soyflour and Hydrogenated Vegetable Oil	14,500*
Sodium Caseinate	0
S.C. Enhancer	5,000
Imitation Milk Base	300
Sodium Caseinate	400
Kreme-Even Base	100
Promine-D	0
CPC Sodium Caseinate	0

\* Mold growth

The products which were purchased frozen or in aerosol cans were nearly always lower in total plate count than other type products. Powders, such as coffee whiteners or desert toppings, had consistently higher counts, but from gram stains of selected colonies it appeared that microbial flora of these products was gram-positive, spore-forming rods.

Table 5. Fat and Oil Samples from Manufacturers.

Ingredient	S.P.C./gram
Emulsifier	0
Glycerol None Stearate	0
Hydrogenated Coconut Oil	0
Soybean-Cottonseed Margarine Oil	0
Hydrogenated Veg Oils	0
Hydrogenated Veg Oils + Lecithin	0
Hydrogenated Veg Oils	0
Hydrogenated Veg Oils	0
Mono-and-Di Glycerides - Hydrog. Veg Oils	0
Mono-and-Di Glycerides - Hydrog. Meat Fats	0
Glycerol Lacto Esters of Fatty Acids	0
Hydrog. Veg Oils with BHA and BHT	0

Rapid spoilage in foodstuffs is generally caused by microbial changes, and foods with high microbial counts are not of as good quality as comparable foods with low counts. The low moisture content of products sold in dehydrated form will preclude growth of any bacteria present, but these organisms may survive until the product is reconstituted. If conditions are then favorable for growth, contaminating organisms will grow, and in the case of staphylococci enterotoxin may be produced.

For dehydrated protein foods, such as dairy products, the maximum acceptable water content is 3% or less (may go up to 5% in some cases), so there is no possibility for microbial spoilage under these conditions. Most dehydrated foods are not sterile, and the fate of microbial populations after drying has been studied (Higginbottom, 1953). At relative humidities



below 80%, the number of surviving bacteria in milk powder increased with decreasing humidity to maximum survival at about 10% R.H. and then tended to fall again.

The same trend may very likely be true with imitation milk product powders. Complete sterilization is hard to obtain even if strict sanitation is maintained during processing, and a lapse in cleanliness during packaging could allow introduction of organisms. If moisture content of powders is low enough no growth of organisms will occur, but they may survive. At the market or in the home, high humidity may prevail and moisture content of the product could rise to such a point as to allow microbial growth. Very often contaminating organisms will be spore-formers and spores have a much greater chance for survival in a low moisture environment.

The fact that these imitation products can support the growth of food poisoning organisms is well documented (McKinley & Clark, 1964) (Post, et. al., 1961) (Crisley, 1964), and pre-whipped toppings or fillings may also readily support such growth if given the opportunity. Commercially produced whipped cream products sold as "cream puffs" were found capable of supporting the growth of Staph. aureus and being potentially dangerous. These products lose their desirable appearance after being held at warm temperatures, however, and rejection by consumers probably plays a large part in avoiding food poisoning associated with these products.



## SUMMARY

Microbiological examination of imitation and filled milk products was performed on products purchased commercially and on individual components obtained from manufacturers. Examinations were done for standard plate count, coliform count, staphylococci, and salmonellae.

All commercially purchased products were found to be free of staphylococci and Salmonella while only 4 of 84 (4.7%) were found to contain coliforms. Products which were purchased as a powder showed high standard plate counts much more often than products which were purchased frozen, refrigerated, or in aerosol cans.

Components which were fats or oils had standard plate counts of zero, while powdered components had plate counts ranging from zero to 14,500. None of the components were found to contain salmonellae, staphylococci or coliforms.

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MICROBIOLOGICAL STUDY OF IMITATION AND  
FILLED DAIRY PRODUCTS AND THEIR COMPONENTS

by

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## ABSTRACT

Imitation and filled milk products purchased commercially and components used in fabricating these products were examined to determine their microbiological quality.

Standard plate counts, coliform counts, staphylococci isolation and salmonellae isolation were performed on 105 samples.

The majority of commercially purchased products were found to have plate counts of zero and all were found to be free of staphylococci and salmonellae. Only 4 of 84 (4.7%) were found to contain coliforms. Products in powdered form were most likely to show a high plate count, while products which were purchased frozen, refrigerated, or in aerosol form nearly always had plate counts of zero.

Components which were fats or oils were free of bacteria while those which were powders often showed a high standard plate count. None of the 31 components examined were found to contain staphylococci, coliforms or salmonellae.